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Metabotropic glutamate receptor 5, and its trafficking molecules Norbin and Tamalin, are increased in the CA1 hippocampal region of subjects with schizophrenia

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Abstract

Metabotropic glutamate receptor 5 (mGluR5) is involved in hippocampal-dependent learning and memory, which are processes disrupted in schizophrenia. Recent evidence from human genetic and animal studies suggests that the regulation of mGluR5, including its interaction with trafficking molecules, may be altered in the disorder. However there have been no investigations of hippocampal mGluR5 or mGluR5 trafficking molecules in the postmortem schizophrenia brain to confirm this. In the present study, we investigated whether protein expression of mGluR5, as well as Norbin and Tamalin (modulators of mGluR5 signalling and trafficking), might be altered in the schizophrenia brain, using postmortem samples from the hippocampal CA1 region of schizophrenia subjects and matched controls (n = 20/group). Protein levels of mGluR5 (total: 42%, $p < 0.001$; monomer: 25%, $p = 0.011$; dimer: 52%, $p < 0.001$) and mGluR5 trafficking molecules (Norbin: 47%, $p < 0.001$; Tamalin: 34%, $p = 0.009$) were significantly higher in schizophrenia subjects compared to controls. To determine any influence of antipsychotic drug treatment, all proteins were also correlated with lifetime chlorpromazine equivalents in patients, and separately measured in the hippocampus of rats exposed to haloperidol or olanzapine treatment. mGluR5 was negatively correlated with lifetime antipsychotic drug exposure in schizophrenia patients, suggesting antipsychotic drugs could reduce mGluR5 protein in schizophrenia subjects. In contrast, mGluR5 and mGluR5 trafficking molecules were not altered in the hippocampus of antipsychotic drug treated rats. This investigation provides strong support for the hypothesis that mGluR5 is involved in the pathology of schizophrenia, and that alterations to mGluR5 trafficking might contribute to the hippocampal-dependent cognitive dysfunctions associated with this disorder.

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Metabotropic glutamate receptor 5, and its trafficking molecules Norbin and Tamalin, are increased in the CA1 hippocampal region of subjects with schizophrenia

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Abstract

Metabotropic glutamate receptor 5 (mGluR5) is involved in hippocampal-dependent learning and memory, which are processes disrupted in schizophrenia. Recent evidence from human genetic and animal studies suggests that the regulation of mGluR5, including its interaction with trafficking molecules, may be altered in the disorder. However there have been no investigations of hippocampal mGluR5 or mGluR5 trafficking molecules in the postmortem schizophrenia brain to confirm this. In the present study, we investigated whether protein expression of mGluR5, as well as Norbin and Tamalin (modulators ofmGluR5 signalling and trafficking), might be altered in the schizophrenia brain, using postmortem samples from the hippocampal CA1 region of schizophrenia subjects and matched controls (n = 20/group). Protein levels of mGluR5 (total: 42%, $p < 0.001$; monomer: 25%, $p = 0.011$; dimer: 52%, $p < 0.001$) and mGluR5 trafficking molecules (Norbin: 47%, $p < 0.001$; Tamalin: 34%, $p = 0.009$) were significantly higher in schizophrenia subjects compared to controls. To determine any influence of antipsychotic drug treatment, all proteins were also correlated with lifetime chlorpromazine equivalents in patients, and separately measured in the hippocampus of rats exposed to haloperidol or olanzapine treatment. mGluR5 was negatively correlated with lifetime antipsychotic drug exposure in schizophrenia patients, suggesting antipsychotic drugs could reduce mGluR5 protein in schizophrenia subjects. In contrast, mGluR5 andmGluR5 trafficking molecules were not altered in the hippocampus of antipsychotic drug treated rats. This investigation provides strong support for the hypothesis that mGluR5 is involved in the pathology of schizophrenia, and that alterations to mGluR5 trafficking might contribute to the hippocampal-dependent cognitive dysfunctions associated with this disorder.

1. Introduction

Metabotropic glutamate receptor subtype 5 (mGluR5) is a postsynaptic G-protein coupled receptor (GPCR), best known for its ability to modulate postsynaptic currents induced by the ionotropic N-methyl-D-aspartate glutamate receptor (NMDAR; Matosin and Newell, 2013). A substantial body of evidence indicates that mGluR5 is critically involved in hippocampal-dependent learning and memory. Specifically, several studies have reported that hippocampal mGluR5 activity is important for long-term potentiation(LTP) and long-term depression (LTD), molecular mechanisms underpinning synaptic plasticity (see Mukherjee and Manahan-Vaughan, 2013). *mGluR5* knockout mice display reduced LTP, specifically in the hippocampal cornu ammonis 1 (CA1) region, as well as deficits in spatial learning and memory (Lu et al., 1997). Chronic mGluR5 antagonism in rats similarly induces deficits in LTP in the CA1 region, which correlates with reduced hippocampal mGluR5 protein levels and poorer working and reference memory (Manahan-Vaughan and Braunewell, 2005). Accordingly, mGluR5 positive allosteric modulators (PAMs) enhance the balance of hippocampal LTP and LTD at CA1 synapses, and consequently improve spatial learning in mice (Ayala et al., 2009). Notably, very recent evidence suggests localisation of mGluR5 in this region might modulate the direction or balance of synaptic plasticity (e.g. LTP versus LTD; Purgert et al., 2014).

Despite extensive examination in animal models, the status of hippocampal mGluR5 protein in patients with schizophrenia is largely unknown. Only one study has previously investigated mGluR5 mRNA expression in the hippocampus of schizophrenia subjects (in a small postmortem cohort of 5 schizophrenia subjects and 6 controls), reporting decreased mGluR5 expression in the parahippocampal gyrus, and no alterations in the dentate gyrus, CA1, CA3 or CA4 (Ohnuma et al., 2000). Based on studies indicating the importance of mGluR5 in synaptic plasticity, including reports that suggest mGluR5 deficits lead to schizophrenia-like cognitive dysfunctions, it is important to extend on these works and thoroughly investigate whether hippocampal mGluR5 is altered in schizophrenia, specifically at the protein level, as this might contribute to the manifestation of learning and memory deficits observed in patients with schizophrenia.

Accumulating evidence indicates the importance of mGluR5 protein–protein interactions in the regulation of mGluR5 trafficking, internalisation and signalling (Enz, 2007). The first discovered modulator of mGluR5 was Homer1 (Brakeman et al., 1997), which has since been extensively studied in the context of schizophrenia (Szumlinski et al., 2006). Both Homer1 protein and gene are reported as altered in, or associated with, schizophrenia (Engmann et al., 2011 and Spellmann et al., 2011). However, in recent years, other molecules that modulate mGluR5 localisation and traficking have become apparent. The neuron-specific protein Norbin (neurochondrin) plays a critical role in mGluR5 localisation, and positively regulates mGluR5 signalling. Interestingly, *Norbin* knockout was found to reduce mGluR5-dependent LTP and abolished LTD in CA1 synapses (Wang et al., 2009). In cultured mouse hippocampal CA1/CA3 neurons, the multiscaffold protein Tamalin also proved critical to

mGluR5 neuritic localisation processes (Kitano et al., 2002) and deletion of the Tamalin binding site on mGluR5 induced mGluR5 internalisation in cellular assays (Timms et al., 2013). Furthermore, evidence from a schizophrenia pedigree suggests that mGluR5/Tamalin interactions might be disrupted in the disorder (Timms et al., 2013). As mGluR5 localisation appears to impact on mGluR5 functions (Purgert et al., 2014), it is important to consider the possibility of altered mGluR5 trafficking in the context of schizophrenia. However, the status of these mGluR5 trafficking molecules in any neuropsychiatric pathology, including schizophrenia, has not been assessed.

In the present study, we therefore determined whether mGluR5 protein levels are altered in postmortem samples from the CA1 hippocampal region of schizophrenia subjects compared to healthy controls (n = 20/group). We further examined protein levels of the mGluR5 trafficking molecules Norbin and Tamalin, which collectively play an important role in mGluR5 trafficking, internalisation and signalling (Kitano et al., 2003 and Wang et al., 2009). The CA1 region was chosen for examination due to evidence that mGluR5-mediated LTP might be specific to CA1 synapses (Lu et al., 1997). Subsequently, we ascertained whether any observed changes in protein levels of mGluR5 or its trafficking molecules were influenced by antipsychotic drug (APD) treatment. For this purpose, we treated rats with first- and second-generation APDs (haloperidol and olanzapine respectively) and measured hippocampal protein levels of mGluR5, Norbin and Tamalin.

2. Materials and methods

2.1. Human postmortem brain samples and tissue preparation

Postmortem human CA1 samples from 20 control (no history of psychiatric diagnosis) and 20 schizophrenia subjects, diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV), were obtained from the New South Wales Brain Bank Network (Sydney, Australia). Subjects were matched according to tissue pH, postmortem interval, RNA integrity number (RIN) and age at death (Table 1). APD treatment premortem was standardised to a lifetime chlorpromazine equivalent for each patient, and antidepressant drug treatment history was specified on a qualitative scale (i.e. yes/no). The samples analysed in this study were derived from a cohort we previously used to examine mGluR5 (Brodmann's Area 46, n = 37/group; Matosin et al., 2013). This work was approved by the Human Research Ethics Committees at the University of Wollongong (HE99/222) and the University of New South Wales (HREC 07261). Anatomical identification and preparation of this CA1 tissue have been previously described in detail (Fernandez-Enright et al., 2014).

Table 1. Demographic and clinical characteristics of the postmortem cohort.

	CA1 (n = 20/group)	
	Control	Schizophrenia
Brain pH	6.6 ± 0.03	6.6 ± 0.3
Postmortem interval (hours)	26.1 ± 12.8	28.8 ± 14.1
RNA integrity number	7.2 ± 0.7	7.2 ± 0.5
Age at death (years)	58.2 ± 12.6	55.5 ± 13.5
Gender	2 F, 18 M	9 F, 11 M
Hemisphere	13 R, 7 L	10 R, 10 L
Age of disease onset (years)	–	23.5 ± 6.8
Duration of illness (years)	–	32.05 ± 13.7
Lifetime antipsychotic drug medication[a]	–	668 ± 421
Antidepressant history (yes/no)	–	12

Abbreviations: DLPFC: dorsolateral prefrontal cortex; F: female; L: left; M: male; R: right. Data are expressed as mean ± standard deviation

[a]Standardised chlorpromazine equivalent, mg.

2.2. Animal housing, treatment and tissue preparation

To assess the influence of haloperidol and olanzapine on mGluR5 and mGluR5 endogenous regulator expression, rats were treated with APD medication as previously described (Matosin et al., 2013). Briefly, adult (10 weeks of age) male Sprague-Dawley rats were purchased from the Animal Resource Centre (Perth, Australia). Rats were separated into groups according to the 3 treatments and 3 treatment-durations (9 groups; n = 6/group). Animals were fed prepared food pellets (sweet cookie dough containing 62% carbohydrate, 22% protein, 10% vitamins, 6% fibre and minerals) 3 times/day at 8 hour intervals, containing either typical antipsychotic, haloperidol (0.1 mg/kg body weight; daily total 0.3 mg/kg), or atypical antipsychotic, olanzapine (1 mg/kg body weight; daily total 3 mg/kg), or vehicle control (no drug), for short term (8 days), medium term (15 days) and long term (35 days) durations. Food and water were available ad libitum, and consumption of food pellets was visually validated. APD doses were chosen to model a clinical setting (Han et al., 2008 and Weston-Green et al., 2012).

Forty-eight hours after final treatment, rats were euthanised using carbon dioxide asphyxiation. The hippocampus (Bregma − 2.30 to − 5.20; total of dorsal and ventral regions) was dissected on ice, snap frozen in liquid nitrogen and then stored at − 80 °C until use. Tissue was homogenised in NP-40 lysis buffer (Invitrogen, Australia), containing ß-glycerophosphate and pheylmethanesulfonylfluoride and protease inhibitor cocktail (Sigma). Protein concentration was determined by DC assay (Bio-Rad), according to the manufacturer's instructions. All animal experiments in this study were approved by the University of Wollongong Animal Ethics Committee

(AE10/18) and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.3. Immunoblot analyses

Immunoblotting was performed on total homogenates as described previously (Matosin et al., 2013), with minor modifications optimised for the measurement of each protein of interest. Each lane was loaded with 5 µg (human) or 10 µg (animal) of total protein. These protein concentrations were determined to be in the linear range of detection (data not shown). Relative protein densities were determined by immunoblot analyses using polyclonal antibodies as previously reported: mGluR5 [ABCAM ab27190; human 1:250, rat 1:500 (Matosin et al., 2013)], Norbin [ABCAM ab130507; human and rat 1:500], and Tamalin [ABCAM ab30576; human 1:100, rat 1:1000 (Tai et al., 2010)]. mGluR5 monomer was detected at 135 kDa whilst the mGluR5 dimer was detected at 270 kDa. Individual bands were totalled before normalisation to respective ß-actin and pooled samples to gain a measure of total mGluR5. Samples were visualised using an enhanced chemiluminescent detection kit (Bio-Rad). Band density was detected by the Gel Doc 2200 Pro (Carestream Molecular Imaging, USA) and quantified with Carestream MI software (v 5.0.4.44, Carestream Molecular Imaging). All bands were within the limits of saturation. Protein measures were subsequently normalised to their respective ß-actin density. Experiments and quantification were performed blind to diagnosis (human) or treatment group (rat).

2.4. Statistical analyses

Statistical analyses were performed with SPSS software (v19.0). The level of significance was set to $p < 0.05$ and data are presented as mean ± SEM.

2.4.1. Human brain statistical analyses

Distributions of mGluR5 total measures (but not individual monomer or dimer measures) as well as Norbin and Tamalin were skewed to the right (Kolmogorov–Smirnov; $d = 0.152–0.246$, $p < 0.001$). Normalised distribution for these proteins was therefore achieved by transforming to the natural logarithm of the relative protein values. Outliers were screened as mean ± 2 standard deviations and removed. Analyses of Variance (ANOVA) were used to detect differences in protein expression between diagnostic groups (schizophrenia/control) as well as gender (male/female), hemisphere (left/right) and antidepressant history (yes/no) for each target. Spearman's correlations were used to determine whether sample characteristics (Table 2) were associated with protein measures, and to examine if additional measures of disease characteristics were correlated specifically with the schizophrenia group (lifetime APD history, age of disease onset and duration of illness). Analyses of covariance (ANCOVA) for diagnostic effects on protein expression were subsequently performed, accounting for sample characteristics that were associated with protein measures. Further Spearman's

correlations were performed to determine the strength of associations between mGluR5 measures and its trafficking molecules in schizophrenia compared to control subjects.

2.4.2. Animal brain statistical analyses

Values for mGluR5 (total) and Norbin were skewed to the right (Kolmogorov–Smirnov; $d = 0.103–0.169$, $p < 0.001$), and thus were normalised by converting to the natural logarithm. Protein differences between treatment (haloperidol/olanzapine/vehicle) and duration (short term/medium term/long term) were analysed by two-way ANOVA.

3. Results

3.1. Protein levels of mGluR5 in schizophrenia subjects compared to controls

We detected a robust increase in mGluR5 protein levels in the CA1 region of schizophrenia subjects compared to controls (total: + 42%, $F_{1,37} = 138.579$, $p < 0.001$;monomer: + 25%, $F_{1,37} = 7.194$, $p = 0.011$; dimer: + 52%, $F_{1,37} = 51.705$, $p < 0.001$;Fig. 1). Demographic and clinical measures that correlated significantly with mGluR5 protein levels (Table 2) were assessed for their influence on the data. After co-varying for freezer storage time and brain pH, which significantly correlated with mGluR5 (total and dimer), significant differences between schizophrenia and control were maintained (mGluR5 total: $F_{1,35} = 172.974$, $p < 0.001$; mGluR5 dimer: $F_{1,35} = 59.433$, $p < 0.001$). An effect of gender was observed, whereby mGluR5 total protein was decreased in male subjects independent of diagnosis ($F_{1,37} = 5.357$, $p = 0.026$; − 35.5%); however, there was no gender-specific difference in mGluR5 protein levels within the schizophrenia or control groups, and no hemispheric differences in mGluR5 protein expression

3.2. Protein levels of mGluR5 trafficking molecules, Norbin and Tamalin, in schizophrenia subjects compared to controls

Norbin and Tamalin proteins were identified at the expected molecular weights (79 kDa and 96 kDa respectively; Fig. 2). Protein levels of these mGluR5 trafficking molecules were significantly increased in schizophrenia (Fig. 2). Specifically, Norbin was increased by 46.7% ($F_{1,37} = 19.600$, $p < 0.001$) and Tamalin by 34.0% ($F_{1,37} = 7.630$, $p = 0.009$). There were no effects of gender or hemisphere on Norbin or Tamalin. Considering the nature of our results whereby all measured proteins of interest were increased, we further analysed protein levels of α-tubulin as a negative control to eliminate the possibility of a global protein increase within the schizophrenia samples. Protein levels of α-tubulin (05-829, Millipore) were not significantly different between schizophrenia and control subjects ($F_{1,37} = 1.991$, $p = 0.167$), validating our findings of increased mGluR5 and mGluR5 trafficking molecules.

3.3. Effects of clinical and demographic variables, and premortem medication estimates on protein measures

Spearman's correlations were performed to determine any relationships between demographic/clinical variables and protein levels of mGluR5 (total, monomer and dimer), Norbin and Tamalin (Table 2). There was a significant correlation between mGluR5 total protein and brain pH in all subjects, but this was not maintained in the individual diagnostic groups. There was an effect of freezer storage time on mGluR5 total and dimer levels. A significant negative correlation was observed between lifetime antipsychotic drug dose and mGluR5 monomer levels in schizophrenia subjects; this association was also trending for mGluR5 dimer levels. APD measures did not correlate with Norbin or Tamalin protein levels. Lastly, there were no effects of antidepressant medication (yes/no; $F_{17–18} \geq 0.015$, $p \geq 0.189$) on mGluR5, Norbin or Tamalin protein levels.

3.4. Relationships of mGluR5 protein (total, monomer and dimer) with Norbin and Tamalin in schizophrenia subjects compared to controls

Spearman's correlations were performed to determine the presence or loss of associations between mGluR5 and its trafficking molecules in control and schizophrenia subjects (Table 3). Although mGluR5 monomer and dimer levels were strongly and positively correlated in both control and schizophrenia subjects ($r \geq 0.790$, $p \leq 0.001$), there were no correlations between measures of mGluR5 (total, monomer or dimer) and its trafficking molecules in control or schizophrenia groups.

3.5. Protein levels of mGluR5 and its trafficking molecules in the hippocampus of APD treated rats

To assess the effects of current antipsychotic medications on the mGluR5 system, two-way ANOVA were used to compare the effects of haloperidol and olanzapine treatment (8, 16 or 36 days) on protein levels of mGluR5 (total, monomer or dimer), Norbin and Tamalin proteins in the hippocampus. There were no significant effects of olanzapine or haloperidol treatment overall or following the specific treatment periods ($F \geq 0.229$, $p \geq 0.296$).

4. Discussion

In the present study, we provide the first evidence that protein expression of mGluR5 is significantly higher (total: 42%; monomer: 25%; dimer: 52%) in the hippocampal CA1 region of schizophrenia subjects relative to healthy controls. Schizophrenia subjects also show a marked increase in Norbin, and Tamalin proteins (47% and 34% respectively), which are endogenous regulators of mGluR5 signalling and trafficking not before analysed in neuropsychiatric pathology. In addition, we found no influence of APD treatment on mGluR5 regulatory proteins in human subjects, however a negative association between lifetime APD dose and mGluR5 protein expression levels was observed in schizophrenia subjects. There were no alterations in mGluR5 or its trafficking molecules in the hippocampus in response to APD treatment in our animal model.

mGluR5 activation in CA1 is critically involved in cognitive functions via mGluR5-mediated modulation of synaptic plasticity (Mukherjee and Manahan-Vaughan, 2013). *mGluR5* knockout mice display deficits in CA1-dependent LTP, coupled with deficits in spatial learning and memory (Lu et al., 1997). mGluR5 PAMs, which upregulate mGluR5 activity, have been reported to enhance hippocampal-dependent LTP and LTD in the CA1 region, congruent with improved performance in the Morris water maze, a measure of hippocampal-dependent spatial learning (Ayala et al., 2009). mGluR5-dependent differences in spatial learning in rats correlated with measures of synaptic plasticity in CA1 and with mGluR5 protein levels (Manahan-Vaughan and Braunewell, 2005). As cognitive dysfunction is a common symptom among schizophrenia patients (Harvey, 2013), the observed increase in mGluR5 in the present study likely represents a compensatory upregulation for mGluR5 dysfunction in this region or in response to wider deficits in glutamatergic signalling. As recently reviewed by Hu et al. (2014), reductions in the ionotropic glutamatergic receptors, NMDA (Vrajová et al., 2010), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA; Eastwood et al., 1995 and Eastwood et al., 1997) and kainate (Benes et al., 2001), have been reported in the hippocampus. Although one study also reported no changes in these receptors (Breese et al., 1995), it is notable that there has been no increase detected.

In support of a deficit in mGluR5, Timms et al. recently reported the presence of a missense mutation in the *mGluR5* gene in a schizophrenia pedigree, transmitting to the extracellular domain of the mGluR5 protein and likely altering mGluR5 sensitivity to glutamate (Timms et al., 2013). Additionally, the authors reported a frame-shift mutation (P1148fs) that caused deletion of the Tamalin-binding site on the carboxyl tail of mGluR5, disrupting Tamalin/mGluR5 interactions and increasing mGluR5 internalisation in primary hippocampal cultures (Timms et al., 2013). We have also recently discovered the significant association of a single nucleotide polymorphism, located in the regulatory3′ untranslated region (highly involved in regulation of protein–protein interactions) within the *GRM5* gene, and schizophrenia in a Caucasian case–control population (unpublished results). Considering this evidence, Tamalin (but also Norbin) proteins might be upregulated in schizophrenia to compensate for reduced protein–protein interactions. Whilst Norbin has been previously demonstrated to positively regulate mGluR5 signalling (Wang et al., 2009), both Norbin and Tamalin are also critical regulators of mGluR5 trafficking (Kitano et al., 2002 and Wang et al., 2009). Therefore our finding of increased levels of these proteins further support that mGluR5 signalling might be altered in the CA1 region of schizophrenia subjects, and these trafficking molecules are upregulated in an attempt to compensate for alterations to mGluR5 signalling and trafficking.

Although it is accepted that mGluR5 is expressed on the postsynaptic neuronal membrane, increasing evidence supports that the majority (60–90%) of mGluR5 is distributed at intracellular locations, including the endoplasmic reticulum membrane and nuclear membrane (Jong et al., 2014). Depending on its cellular location, mGluR5 reportedly activates different signalling cascades leading to unique

cellular responses (Jong et al., 2014). Accordingly, intracellular and extracellular mGluR5 differentially mediate synaptic plasticity in the CA1: cell surface receptors contribute to both LTD and LTP, whereas activation of intracellular mGluR5 is involved in LTD only (Purgert et al., 2014). Considering our finding of alterations to proteins that regulate mGluR5 movement to/from neuronal membranes, using total homogenate preparations, it will be important to determine whether the increase in mGluR5 observed in this study represents increases in intracellular or cell-surface distribution of mGluR5, or both.

Premortem APD exposure can influence molecular measures in postmortem tissue analyses. In the present study, estimated lifetime APD exposure was negatively correlated with mGluR5 monomer levels and a correlation approaching significance was observed with mGluR5 dimers. This finding suggests an influence of APD on mGluR5 protein levels in the CA1 whereby APDs might act to reduce mGluR5 protein levels in the CA1. However considering we conversely detected increased mGluR5 protein levels in this region, and we found no influence of APD treatment on mGluR5 in the hippocampus of APD treated rats, it is unlikely that APD exposure is confounding the results of our study. Furthermore, we additionally report no association between Norbin, Tamalin, and APDs in our present human or rat studies, and we have previously reported that mGluR5 binding was not associated with APD exposure in the hippocampus (Matosin et al., 2013). Thus although an interaction is unlikely, additional studies are required to explore the mechanism underlying a potential association. Further investigation of the consequences of APD treatment on the mGluR5 system in animal models of schizophrenia would be valuable.

mGluR5 is a promising therapeutic target for the treatment of cognitive deficits in schizophrenia (Matosin and Newell, 2013). The present study reports that mGluR5 and mGluR5 trafficking molecules are altered in the CA1 region in subjects with schizophrenia, suggesting that mGluR5 signalling and trafficking is altered. It will be important for future studies to elucidate the exact mechanisms underlying these alterations and their implications for novel mGluR5-targeting therapeutics, to determine how to best target mGluR5 (if at all) in a manner that will be therapeutic in the context of pathological alterations. For example, altered cell surface expression might limit available targets for drugs aimed at mGluR5 and the use of pharmacological chaperones to attenuate mGluR5 trafficking deficits may be beneficial (Ulloa-Aguirre and Conn, 2011), whilst insensitivity to glutamate (as proposed by Timms et al., 2013) may positively or negatively affect PAMs that rely on simultaneous glutamate activation.

We conclude that hippocampal CA1 protein levels of mGluR5 and mGluR5 trafficking molecules are increased in the pathology of schizophrenia. We hypothesise that this may be related to a functional deficit within mGluR5, whereby glutamate sensitivity, protein–protein interactions or mGluR5 localisation are affected. Due to the prominent role of hippocampal mGluR5 in the emergence of learning and memory deficits, we further suggest that alterations to mGluR5 in CA1 may be

associated with an imbalance of LTD:LTP, thus contributing to cognitive dysfunctions that are observed in schizophrenia patients. However, future studies investigating the correlation between hippocampal mGluR5 expression and cognition in humans are required to confirm this hypothesis.

Role of funding source

The funding sources had no role in this study, including study design, data collection and publication decisions.

Contributors

All authors contributed to the study design and have contributed to and approved the final manuscript.

Conflict of interest statement

All authors declare that they have no conflicts of interest.

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Figures and Tables

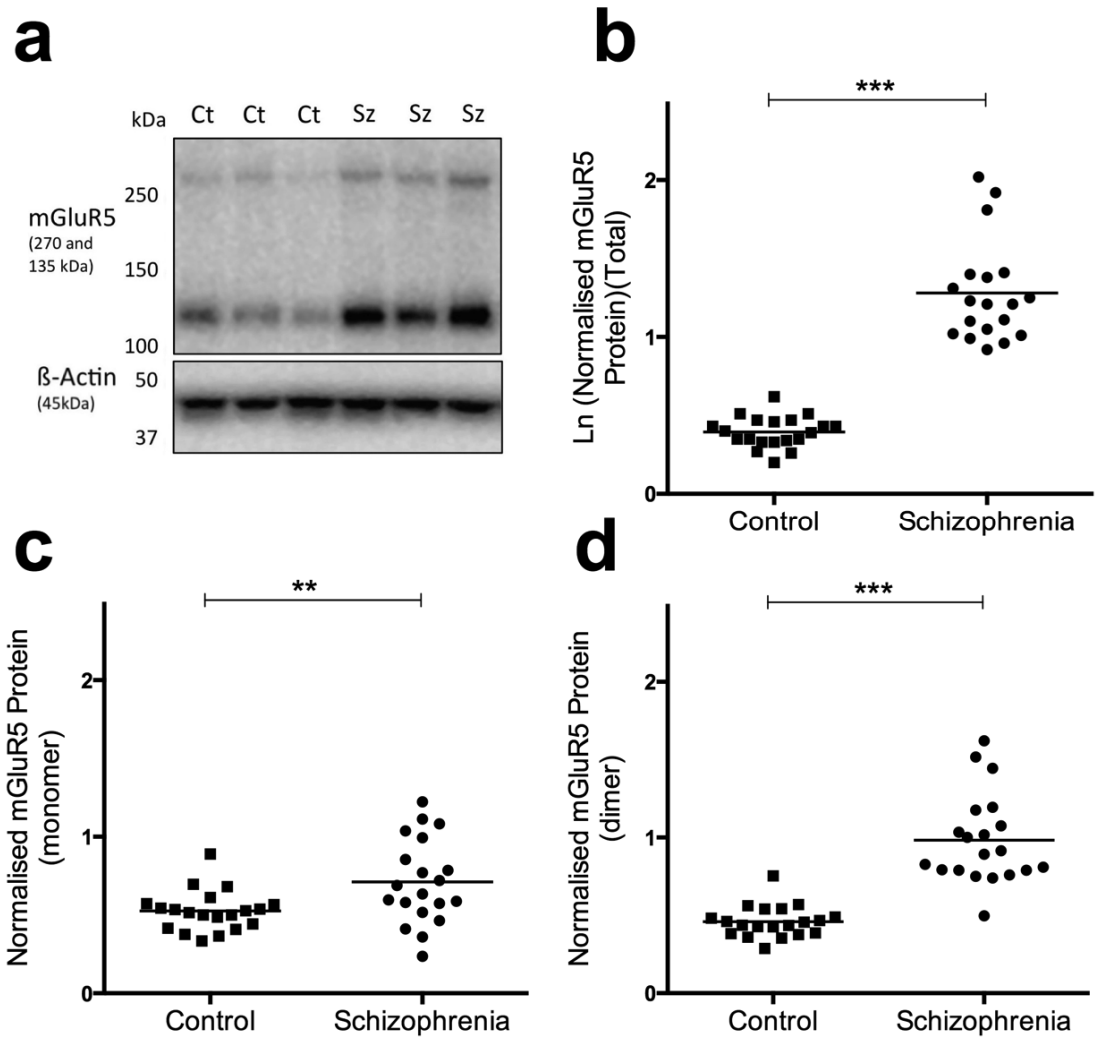

Figure 1. Protein levels of mGluR5 in the CA1 region of subjects with schizophrenia and matched controls. (a) Representative immunoblots, and normalised levels of mGluR5 **(b)** total, **(c)** monomer, and **(d)** dimer protein measures, in schizophrenia subjects compared to controls. **Abbreviations:** Ct: control; kDa: kilodaltons; Ln: natural logarithm; mGluR5: metabotropic glutamate receptor 5; Sz: schizophrenia. ***P<0.05, **P<0.01, ***P<0.001.**

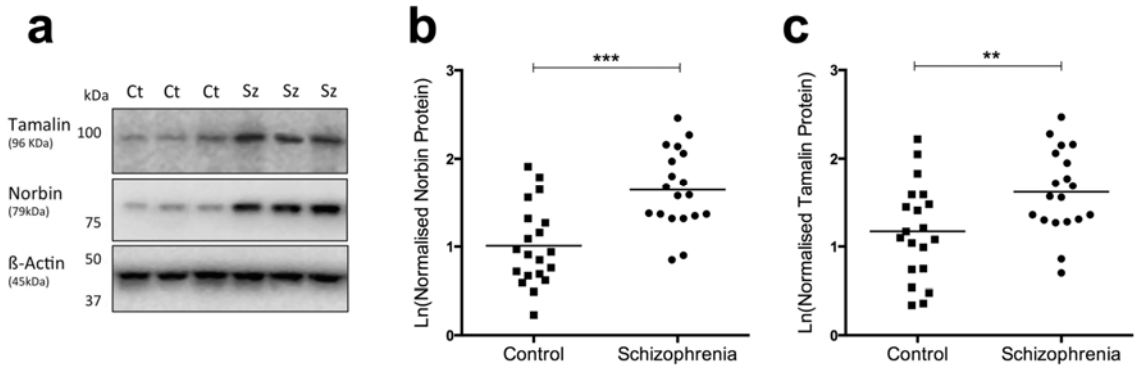

Figure 2. mGluR5 trafficking molecules Norbin and Tamalin in the CA1 region of subjects with schizophrenia. (a) Representative immunoblots, and normalised protein levels of mGluR5 trafficking molecules **(b)** Norbin and **(c)** Tamalin in the CA1 region of subjects with schizophrenia compared to matched controls. **Abbreviations:** Ct: control; kDa: kilodaltons; Ln: natural logarithm; Sz: schizophrenia. ***P<0.05, **P<0.01, ***P<0.001.**

Table 1. Demographic and clinical characteristics of the postmortem cohort.

	CA1 (n=20/group)	
	Control	Schizophrenia
Brain pH	6.6 ± 0.03	6.6 ± 0.3
Postmortem interval (hours)	26.1 ± 12.8	28.8 ± 14.1
RNA integrity number	7.2 ± 0.7	7.2 ± 0.5
Age at Death (years)	58.2 ± 12.6	55.5 ± 13.5
Gender	2 F, 18 M	9 F, 11 M
Hemisphere	13 R, 7L	10R, 10L
Age of disease onset (years)	-	23.5 ± 6.8
Duration of illness (years)	-	32.05 ± 13.7
Lifetime antipsychotic drug medication*	-	668 ± 421
Antidepressant history (yes/no)	-	12

*Standardised chlorpromazine equivalent, mg**.**

Abbreviations: DLPFC: dorsolateral prefrontal cortex; F: female; L: left; M: male; R: right. Data are expressed as mean ± standard deviation

Table 2. Spearman's Correlations for continuous clinical and demographic variables and protein levels of mGluR5 total, monomer and dimer, and mGluR5 trafficking molecules Norbin and Tamalin, in the hippocampal CA1 region. Significant values ($p<0.05$) are highlighted in bold.

Variable	All subjects					Controls					Schizophrenia				
	mGluR5 (total)	**mGluR5 (monomer)**	**mGluR5 (dimer)**	**Norbin**	**Tamalin**	**mGluR5 (total)**	**mGluR5 (monomer)**	**mGluR5 (dimer)**	**Norbin**	**Tamalin**	**mGluR5 (total)**	**mGluR5 (monomer)**	**mGluR5 (dimer)**	**Norbin**	**Tamalin**
Brain pH	**r = -0.355 p = 0.026**	r = -0.001 p = 0.993	r =-0.088 p =0.593	r = 0.015 p = 0.929	r = 0.026 p = 0.874	r = -0.304 p = 0.192	r = -0.040 p = 0.867	r = -0.134 p = 0.573	r = 0.142 p = 0.551	r = 0.127 p = 0.593	r = -0.448 p = 0.055	r = -0.084 p = 0.732	r =-0.116 p = 0.497	r = -0.170 p = 0.486	r = -0.147 p = 0.549
Age at Death	r = -0.028 p = 0.868	r = -0.154 p = 0.349	r =-0.164 p = 0.320	r = -0.096 p = 0.561	r = -0.096 p = 0.560	r = -0.188 p = 0.427	r = -0.087 p = 0.716	r = 0.136 p = 0.569	r = -0.068 p = 0.777	r = -0.061 p = 0.798	r = 0.287 p = 0.233	r = -0.184 p =0.452	r = -0.211 p = 0.386	r = -0.199 p = 0.414	r = -0.187 p = 0.444
Postmortem interval	r = -0.167 p = 0.309	r = 0.100 p = 0.546	r = 0.170 p = 0.302	r = -0.051 p = 0.758	r = -0.085 p = 0.606	r = -0.199 p = 0.400	r = -0.199 p = 0.401	r = -0.118 p = 0.620	r = 0.113 p = 0.635	r = 0.049 p = 0.838	r = -0.328 p = 0.170	r = 0.250 p = 0.302	r = 0.359 p = 0.131	r = -0.158 p = 0.517	r = -0.170 p = 0.487
Freezer storage time	**r = 0.356 p = 0.026**	r =0.243 p =0.136	**r = 0.347 p =0.030**	r = 0.173 p = 0.292	r = 0.159 p = 0.334	r = 0.274 p = 0.243	r = 0.335 p = 0.149	r = 0.332 p = 0.152	r = 0.219 p = 0.354	r = 0.162 p = 0.496	**r = 0.531 p = 0.019**	r = 0.207 p =0.395	r = 0.354 p = 0.137	r = 0.220 p = 0.365	r = 0.210 p = 0.389
Brain weight	r = -0.144 p = 0.383	r = -0.039 p = 0.815	r = -0.232 p = 0.156	r = -0.258 p = 0.113	r = -0.176 p = 0.283	r = -0.267 p = 0.255	r = -0.083 p = 0.729	r = -0.170 p =0.473	r = -0.123 p = 0.606	r = -0.042 p = 0.860	r = 0.030 p = 0.903	r = -0.069 p = 0.780	r = -0.194 p = 0.425	r = -0.145 p = 0.553	r = -0.142 p = 0.563
Age of disease onset	-	-	-	-	-	-	-	-	-	-	r = 0.259 p = 0.285	r = 0.241 p = 0.321	r = 0.156 p = 0.523	r = 0.051 p = 0.837	r = 0.078 p = 0.750
Duration of illness	-	-	-	-	-	-	-	-	-	-	r = 0.199 p = 0.415	r = -0.234 p = 0.336	r = -0.232 p = 0.339	r = -0.231 p = 0.341	r = -0.224 p = 0.357
Lifetime antipsychotic drug intake*	-	-	-	-	-	-	-	-	-	-	r = -0.160 p = 0.514	**r = -0.502 p = 0.029**	r = -0.426 p = 0.069	r = -0.251 p = 0.300	r = -0.256 p = 0.290

* Standardised chlorpromazine equivalent (mg)

Table 3. Spearman's correlations for associations between protein levels of mGluR5 (total, monomer and dimer) and novel mGluR5 signalling partners Norbin and Tamalin, within (A) control and (B) schizophrenia subjects. Significant values ($p<0.05$) are highlighted in bold.

A. Control	*mGluR5 total*	*mGluR5 monomer*	*mGluR5 dimer*
mGluR5 (total)	-	r = 0.066	r = 0.102
	-	p = 0.782	p = 0.668
mGluR5 (monomer)	r = 0.066	-	**r = 0.853**
	p = 0.782	-	**p < 0.001**
mGluR5 (dimer)	r = 0.102	**r = 0.853**	-
	p = 0.668	**p < 0.001**	-
Norbin	r = -0.036	r = 0.274	r = 0.266
	p = 0.980	p = 0.243	p = 0.257
Tamalin	r = 0.017	r = 0.334	r = 0.292
	p = 0.945	p = 0.150	p = 0.212

B. Schizophrenia	*mGluR5 total*	*mGluR5 monomer*	*mGluR5 dimer*
mGluR5(total)	-	r = 0.140	r = 0.137
	-	p = 0.557	p = 0.566
mGluR5(monomer)	r = 0.140	-	**r = 0.790**
	p = 0.557	-	**p < 0.001**
mGluR5 (dimer)	r = 0.137	**r = 0.790**	-
	p = 0.566	**p < 0.001**	-
Norbin	r = 0.037	r = 0.001	r = 0.064
	p = 0.876	p = 0.996	p = 0.788
Tamalin	r = 0.027	r = 0.038	r = 0.029
	p = 0.910	p = 0.875	p = 0.902